Detection of Macrophage Migration Inhibitory Factor by Monoclonal Antibody in Sézary Syndrome

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We have reported previously on the generation of a monoclonal antibody against human macrophage migration inhibitory factor (MIF), which is a mediator of cellular immunity. Macrophage migration inhibitory factor activity in the migration assay was closely correlated with antibody reactivity. Using this antibody called 1C5/B, we are now able to study the expression of MIF in situ. Here, we report on the detection of MIF in blood lymphocytes and skin of a patient with a leukemic cutaneous T-cell lymphoma with the characteristics of Sézary syndrome. Ninety percent of the patient's Ficoll Hypaque-isolated peripheral white blood cells were of the helper phenotype. By conventional immunoperoxidase method, 94% reacted strongly positive with the antibody 1C5/B. In contrast, using the immunofluorescence method only 25% reacted positive. This indicates that the majority of the tumor cells did not express the molecule on their membrane but only in the cytoplasma. No other marker, such as interleukin 2 receptor, HLA-DR antigen, or interferon-gamma could be related to the expression of MIF. Also the cellular infiltrate in the skin was composed mainly of T helper cells and reacted positive with 1C5/B. As less than 3% of normal blood lymphocytes reacted with 1C5/B we suggest that the conversion to positivity may be a characteristic feature of the leukemic T-cell phenotype in Sézary syndrome. *J Invest Dermatol 88:670–674, 1987*

Macrophage migration inhibitory factor (MIF) was the first lymphokine to be described and was defined by its inhibitory effect on the migration of macrophages from capillary tubes [1]. Its release by cultured mononuclear cells upon antigen stimulation has been considered to be an equivalent to the state of hypersensitivity in vivo. Relatively little information is available on the production of MIF in vivo and its relation to the physiology and pathology of inflammatory reactions. An MIF-like activity was previously detected in the serum of animals which had been infected with bacillus Calmette-Guérin and of patients with sarcoidosis, leprosy, and Sézary syndrome, a leukemic T-cell lymphoma of helper cell type [2–5]. However, information on the distribution and function of MIF in the organism is lacking. Recently this molecule has been characterized and has been shown to exert profound effects on macrophage differentiation [6,7]. From in vitro experiments it is generally concluded that MIF is produced by T cells when they are stimulated either by antigen or mitogen [1]. Direct identification of the cells that contain MIF has now become possible by a monoclonal antibody, which we prepared against highly purified MIF from mitogen-stimulated human peripheral blood mononuclear cells [8]. We show here that the malignant T cells of a patient with a leukemic cutaneous T-cell lymphoma (CTCL) of the helper type express MIF-related structures.

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Abbreviations:
1C5/B: biotinylated antibody against the 14 kD molecular weight species of human MIF
CTCL: cutaneous T-cell lymphoma
HTLV: human T lymphotropic virus
IFN-γ: gamma interferon
IL-2: interleukin 2
MIF: macrophage migration inhibitory factor

MATERIALS AND METHODS

Case History A 51-year-old woman with a 2-year history of erythroderma and grossly enlarged axillary and groin lymph nodes showed rising peripheral white blood cell counts some months before the investigation. At the time of investigation the total white cell count was 56,000/μl, a differential blood count showed 70% lymphocytes, 2% eosinophils, 2% monocytes, and 16% neutrophils. Most lymphocytes appeared atypical, being larger than normal with prominent nucleoli and approximately 20% had a highly indented nucleus. Lactate dehydrogenase in the serum was strongly elevated (900 units/ml). Anti-HTLV-I, -II antibodies, as evaluated by an enzyme-linked immunosorbent assay were negative. The examination of an enlarged lymph node of the groin (Prof. Dr. K. Lennert, Kiel, F.R.G.) revealed a T-zone lymphoma, in agreement with a Sézary syndrome. By ultrasound investigation abdominal lymph nodes, spleen, and liver were found to be normal. A bone marrow smear showed no infiltration with malignant cells at the time of investigation.

Blood Cells Lymphoid cells were isolated from heparinized blood by Ficoll Hypaque centrifugation (Pharmacia, Uppsala, Sweden) and used for preparation of semithin sections, cytocentrifuge smears, and for flow cytofluorometry with an EPICS V (Coulter Electronics, Inc., Hialeah, Florida). The percentage of cells that were labeled positive by indirect immunofluorescence technique was calculated by the Immuno Software supplied by

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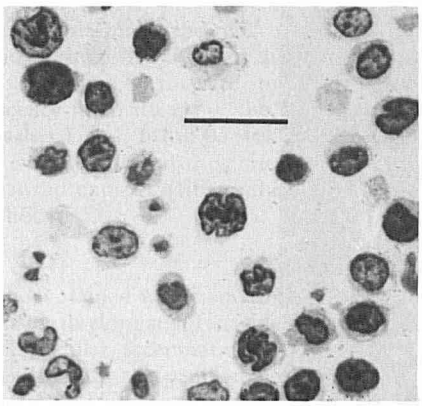

Figure 1. Semithin section of Ficoll Hypaque-isolated peripheral blood cells. Many cells show highly convoluted nuclei and heterochromatin attached to their nuclear membrane. *Bar* = 10 μm. Counterstain: Meyer's haemalaun.

Coulter. For semithin sections the cell button was fixed with glutaraldehyde (4%).

Antibodies Monoclonal antibodies diluted as indicated in the specification sheets of the suppliers were used: Leu 3a, Leu 4, anti-interleukin 2 (IL-2) receptor (Becton Dickinson, Heidelberg, F.R.G.); anti-interferon-gamma (IFN-γ), (code 2067, Celltech, GB), T4, T8, T6 (Coulter Clone, Krefeld, F.R.G.); Ki67 (proliferation marker) (Dakopatts, Hamburg, F.R.G.); OKM1, OKM5 (Ortho Diagnostics, Hamburg, F.R.G.); and D-1-12 for detection of HLA-DR antigen (kindly provided by Dr. S. Carrel, Lausanne, Switzerland). Production procedure and characterization of the monoclonal antibody 1C5 against human MIF were described elsewhere [8]. Briefly, the antibody was generated against a 14 kD species of MIF from human peripheral blood mononuclear cells and binds but does not neutralize MIF [8]. The antibody was biotinylated according to Guesdon et al [9] and diluted 1:400.

Skin Six-millimeter punch biopsies were taken from the forearm and either fixed in buffered formalin for routine histologic investigation or snap-frozen in liquid nitrogen for immunolabeling.

Immunoperoxidase Technique Six-micrometer frozen skin sections were air-dried with acetone (10 min) and preincubated with 0.1% hydrogen peroxide and 1% bovine serum albumin. Incubation with the relevant antibody was performed for 45 min at room temperature. Secondary to the commercial antibodies peroxidase-conjugated goat antimouse IgG F(ab')$_2$ (Dianova, Hamburg, F.R.G.) was applied for 30 min at room temperature. Secondary to the biotinylated 1C5/B antibody peroxidase-conjugated Streptavidin (Amersham, Braunschweig, F.R.G.) was used. All antibodies were diluted with phosphate-buffered saline, containing 1% bovine serum albumin. Peroxidase activity was demonstrated with the 3-amino-9-ethylcarbazole reaction [10]. The preparations were counterstained with Meyer's haemalaun (Merck, Darmstadt, F.R.G.).

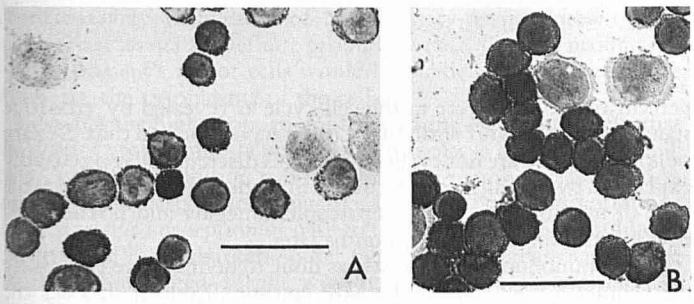

Figure 2. Cytocentrifuge preparations of Ficoll Hypaque-isolated peripheral blood cells from the patient. *Bars* = 30 μm. Counterstain: Meyers haemalaun. *A*, Leukemic tumor cells stain positive for MIF with the 1C5/B antibody. Monocytes are negative at the employed dilution of antibody. *B*, Leukemic tumor cells stain also positive with Leu 3a antibody, characterizing them as T helper cells.

Table I. Percent Mononuclear Peripheral Blood Cells[a] From Cytocentrifuge Preparations That Stained Positive by Immunoperoxidase

T4	T8	Leu 4	1C5 B	Ki67	IL-2-rec	IFN-γ	D-1-12
90	2	93	94	12	1	1	8

[a]Prepared by Ficoll Hypaque, 200 cells were evaluated by the microscope.

Table II. Percent Mononuclear Peripheral Blood Cells That Stained Positive as Detected by EPICS V[a] Analysis

T4	Leu 4	1C5 B	IL-2-rec
94	88	25	0

[a]Mononuclear cells were prepared by Ficoll Hypaque, 30,000 cells were analyzed.

RESULTS

Blood Semithin sections of Ficoll Hypaque preparations from the peripheral blood of the patient revealed many atypical lymphocytes displaying characteristic features of CTCL (Fig 1).

Figure 2*A* depicts cells from cytocentrifuge smears that stained strongly positive for MIF and Fig 2*B* shows that the cells display the T helper phenotype. Monocytes stained negative with 1C5/B at the employed concentrations. Controls using biotinylated mouse

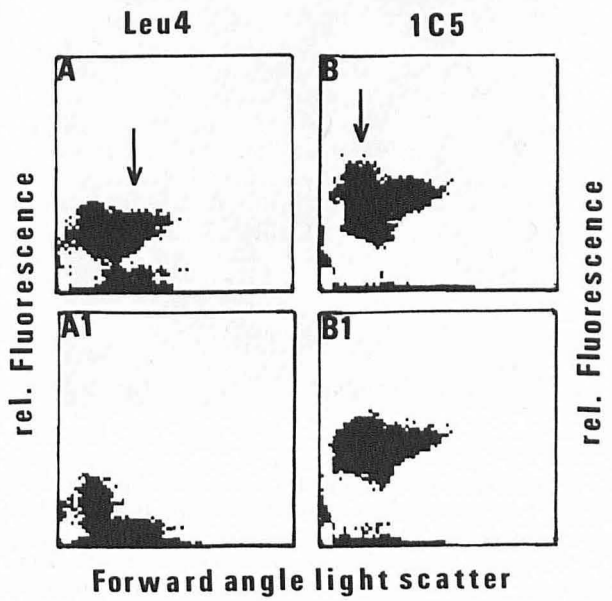

Figure 3. Distribution of membrane-labeled cells according to their fluorescence intensity and size as measured by foreward angle light scatter. 3*A* shows the results with Leu 4 antibody, 3*A1* the corresponding control with mouse IgG. 3*B* shows the results with 1C5/B antibody, 3*B1* the corresponding control with biotinylated mouse IgG. *Arrows* indicate accumulation of cells that stain positive above background. It can be seen that peaks of Leu 4-positive cells and 1C5/B-positive cells do not overlap. This indicates that large Leu 4-positive cells do not express MIF on their membrane.

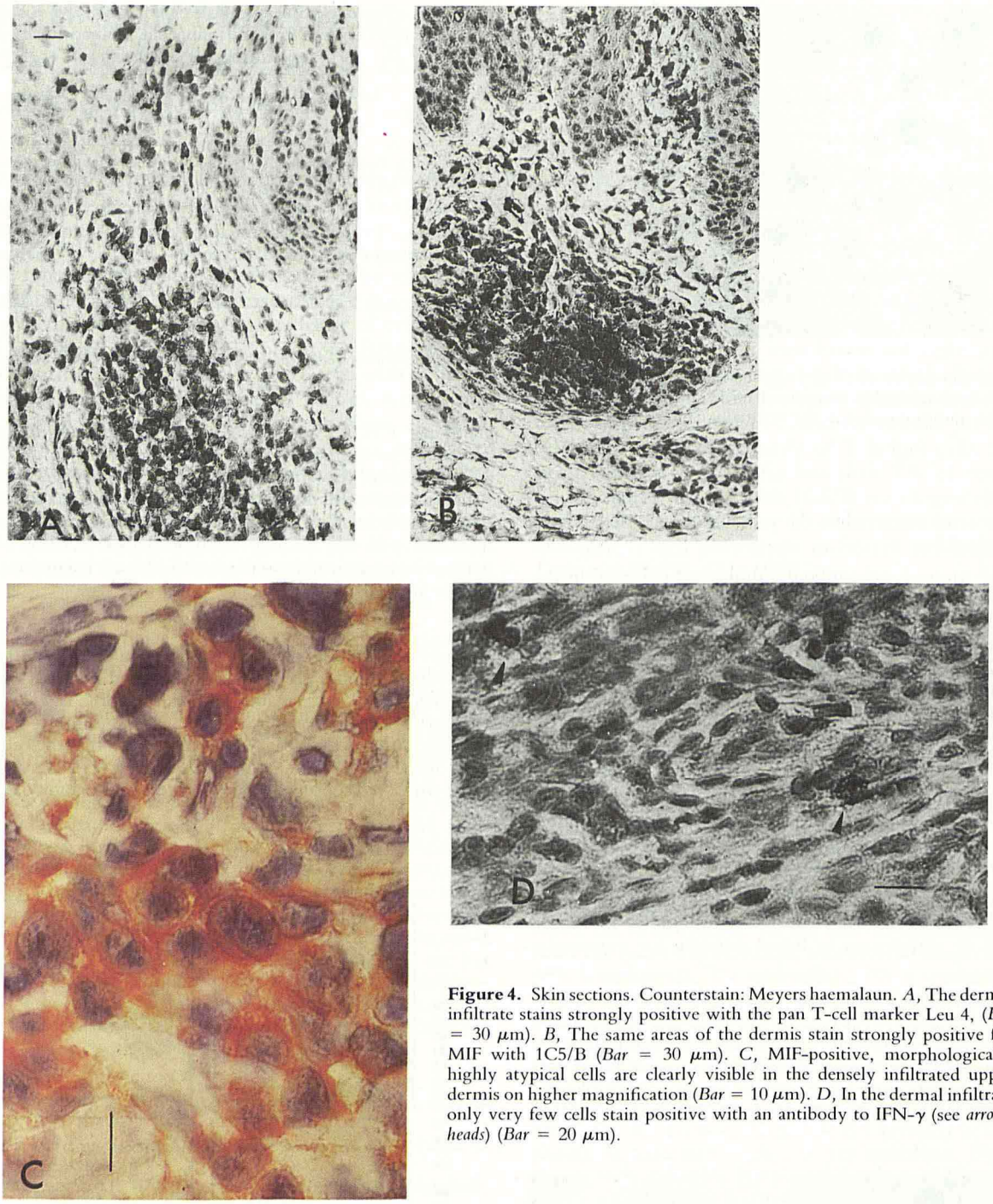

Figure 4. Skin sections. Counterstain: Meyers haemalaun. *A*, The dermal infiltrate stains strongly positive with the pan T-cell marker Leu 4, (*Bar* = 30 μm). *B*, The same areas of the dermis stain strongly positive for MIF with 1C5/B (*Bar* = 30 μm). *C*, MIF-positive, morphologically highly atypical cells are clearly visible in the densely infiltrated upper dermis on higher magnification (*Bar* = 10 μm). *D*, In the dermal infiltrate only very few cells stain positive with an antibody to IFN-γ (see *arrowheads*) (*Bar* = 20 μm).

IgG and Streptavidin were constantly negative. In cytocentrifuge preparation from healthy controls only very few lymphocytes stained positive (≤3%).

Table I shows the percentage of mononuclear cells from cytocentrifuge preparations that stained positive with various other antibodies. Ninety percent of the cells were T cells with the helper phenotype (T4/Leu 3a), which is a typical finding for the Sézary syndrome [11]. Ninety-four percent stained positive for MIF with 1C5/B. In contrast, IFN-γ as well as the IL-2 receptors and HLA-DR antigens D-1-12, which are activation markers, were not expressed. The absence of IL-2 receptors on the circulating tumor cells together with the absence of antibodies to human T lymphotropic virus (HTLV) I and HTLV II clearly distinguished this case from the Japanese form of adult T-cell leukemia [12]. Twelve percent of the cells were in the cell cycle as revealed by positive staining with the Ki67 antibody. Others have reported that Sézary cells in the blood are not cycling [13]. This discrepancy is probably explained by the aggressive course that the disease took at the time of investigation, since hepatosplenomegaly and bone marrow infiltration occurred 3 months later.

An immunofluorescence test was done to define more precisely the cellular localization of the MIF epitope. Table II shows the percentage of mononuclear peripheral blood cells with positive membrane fluorescence as revealed by EPICS V. Again, a high percentage (94%) were positive with the T helper cell marker (T4), indicating its localization on the cell membrane. In contrast, only 25% showed membrane fluorescence with 1C5/B. Comparing this result to the numbers of cells that stained positive by

immunoperoxidase, it becomes apparent that MIF was mainly located in the cytoplasma of the cells.

Figure 3 shows the distribution of the patient's cells according to their size as measured by forward angle light scatter and membrane fluorescence intensity using the 1C5/B antibody and the pan T-cell marker Leu 4. It can be seen that large Leu 4-positive cells did not express MIF on their membrane. From their size, those cells that did express MIF on their membranes resembled normal lymphocytes.

Skin

Histology: Slides stained with hematoxylin-eosin showed thickened epidermis with elongated rete ridges and a dense, band-like infiltrate with nodular accentuation in the upper dermis. The infiltrate extended into the papillary tips with some exocytosis into the epidermis. The lymphocytes were larger than normal, many had a blast-like appearance, others showed highly convoluted nuclei, consistent with the diagnosis of CTCL.

Immunoperoxidase Staining: The infiltrate was mainly composed of T lymphocytes (Fig 4*A*), which almost exclusively carried the T4/Leu 3a marker for T helper cells corresponding to the findings in the peripheral blood. The dermal T cells stained positive with anti-HLA-DR antibody, according to what is known about malignant T cells infiltrating the skin [14]. There was a moderate migration of these cells into the epidermis. In the center of the densely infiltrated areas some dendritic cells stained positive with T6 and probably represented Langerhans cells. In the same area many lymphoid cells expressed the IL-2 receptor as detected by anti-Tac, indicating that they had become activated in the dermis [15]. Macrophages as detected by OKM1 and OKM5 were moderately scattered throughout the dermis. Many cells in the dermis stained positive with 1C5/B even at high dilutions (Fig 4*B, C*). Quantity and pattern of distribution was similar to the atypical Leu 4-positive cells. Controls, including Streptavidin and biotinylated mouse IgG, were negative. Twenty to thirty percent of the infiltrating cells were cycling as they stained positive with Ki67. Some cells migrating into the epidermis were positive with 1C5/B. In contrast, the antibody against IFN-γ detected only very few cells in the dermal infiltrates (Fig 4*D*).

DISCUSSION

Using a monoclonal antibody to MIF we found that circulating and skin-associated leukemic T cells in a patient with advanced Sézary syndrome reacted positive. This finding is of interest because so far MIF-like activity has been detected only in the serum of these patients and in the culture supernatants of peripheral blood mononuclear cells [5]. We cannot decide from our results whether MIF is actively secreted by the tumor cells or released upon cell death. The work on the characterization of the antibody shows that the 1C5 epitope most likely indicates the presence of MIF molecules. When monitoring various separation procedures, the binding curves with 1C5/B antibody overlap qualitatively and quantitatively with those of MIF activity as measured by the migration assay [8]. Definite proof, however, for MIF production by the patient's tumor cells would be the detection of messenger RNA in the cytoplasma of the cells.

Besides the high proportion of circulating leukemic T cells that showed cytoplasmic staining, there was also a cohort of small T cells, which in addition had MIF on their membrane surface. As monocytes clearly stained negative at the antibody concentrations used, passive adsorption of MIF to the cell membrane is unlikely. Except for T-cell markers, other membrane antigens that may be found on activated T cells were negative. The absence of IL-2 receptors and HLA-DR antigens on circulating tumor cells distinguishes the Sézary syndrome from the acute adult T-cell leukemia [12].

In lymphoid organs and peripheral blood of normal control persons only very few lymphocytes have been found to stain positive with 1C5/B (G. Zwadlo et al, unpublished; G. Gerhards et al, unpublished). In contrast, in the elicitation phase of contact eczema many of the lymphocytes infiltrating the skin are MIF-positive (C. Neumann et al, unpublished). Thus, it may be that MIF is preferentially expressed by a distinct subpopulation that has a marked propensity to infiltrate the skin. It may further be speculated that it is this MIF-positive subpopulation that expands in the Sézary syndrome. This hypothesis seems intriguing, as it is increasingly recognized that the skin possesses various important immune mechanisms [16,17]. In two further cases of erythrodermic CTCL but without leukemia we have also found a significant proportion of MIF-positive cells in the dermal infiltrate. In these nonleukemic cases, however, the peripheral blood contained only a few MIF-positive cells. Blastogenic leukemia in CTCL as seen in the patient presented here is an extremely rare, late event in the course of the disease. Further cases have to clarify whether the appearance of MIF-positive tumor cells in the blood is typical for the leukemic stage of the disease.

The functional significance of MIF in CTCL is not clear. As MIF has profound effects on the differentiation of mononuclear phagocytes, it appears appropriate to investigate this cell system in patients with increased MIF expression. Others have suggested that MIF can abrogate cellular immune reactions [18]. Whether the immunosuppression that is seen in patients with CTCL can be related to MIF remains to be investigated.

We also investigated IFN-γ because it is another product of activated T-cell subsets [19]. In contrast to MIF, the blood cells of our patient did not stain positive with an antibody against IFN-γ and accordingly the dermal infiltrate was almost negative. This might be surprising since IFN-γ is secreted upon antigenic stimulation and one of the proposed etiologic factors of CTCL is chronic antigenic stimulation of T cells in the skin [20]. On the other hand, the negativity for IFN-γ might have resulted from functional defects of the malignant T cells, which undoubtedly may occur in CTCL [21].

REFERENCES

1. Bloom BR, Bennet B: Mechanism of a reaction in vitro associated with delayed type hypersensitivity. Science 153:80–82, 1966
2. Salvin SB, Ribi E, Granger L, Youngner JS: Migration inhibitory factor and type II interferon in the circulation of mice sensitized with myobacterial components. J Immunol 114:354–359, 1975
3. Yoshida T, Siltzbach LE, Masih N, Cohen S: Serum macrophage migration inhibitory activity in patients with sarcoidosis. Clin Immunol Immunopathol 13:39–46, 1979
4. Rea TH, Yoshida T: Serum macrophage migration inhibition activity in patients with leprosy. J Invest Dermatol 79:336–339, 1982
5. Yoshida T, Edelson R, Cohen S, Green I: Migration inhibitory activity in serum and cell supernatants in patients with Sézary syndrome. J Immunol 114:915–918, 1975
6. Michels E, Stenzinger W, Sorg C: Functional characteristics of murine macrophages responding to migration inhibitory factors. Eur J Immunol 14:902–905, 1984
7. Sorg C, Michels E, Malorny U, Neumann C: Migration inhibitory factors and macrophage differentiation. Springer Semin Immunopathol 7:311–320, 1984
8. Burmeister G, Tarcsay L, Sorg C: Generation and characterization of a monoclonal antibody (1C5) to human migration inhibitory factor (MIF). Immunobiology 171:461–474, 1986
9. Guesdon JL, Ternynck T, Auramaes S: The use of avidin-biotin interaction in immunoenzymatic techniques. J Histochem Cytochem 27:1131–1139, 1979
10. Graham RC Jr, Lundholm M, Karnovsky MJ: Cytochemical demonstration of peroxidase activity with 3-amino-9-ethylcarbazole. J Histochem Cytochem 13:150–152, 1965
11. Buechner SA, Winkelmann RK, Banks PH: T cells in cutaneous lesions of Sézary syndrome and T cell leukemia. Arch Dermatol 119:895–900, 1983
12. Gootenberg JE, Ruscetti FW, Mier JW, Gadzar A, Gallo RC: Human cutaneous T cell lymphoma and leukemia cell lines produce and respond to T cell growth factor. J Exp Med 154:1403–1418, 1981

13. Schwarzmeier JD, Paietta E, Radaszkiewicz T, Konrad K, Marosi L: Proliferation kinetics of Sézary cells. Blood 57:1049–1054, 1981

14. Haynes BF, Hensley LL, Jegasothy BV: Differentiation of human T lymphocytes. II. Phenotypic difference in skin and blood malignant T cells in cutaneous T cell lymphoma. J Invest Dermatol 78:323–326, 1982

15. Uchiyama T, Broder S, Waldmann TA: A monoclonal antibody (Tac) reactive with activated and functionally mature T cells. 1. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. J Immunol 126:1393–1397, 1981

16. Stingl G, Tamaki K, Katz SI: Origin and function of epidermal Langerhans cells. Immunol Rev 53:149–174, 1980

17. Luger TA, Stadler BM, Katz SI, Oppenheim JJ: Epidermal cell (keratinocyte) derived thymocyte activating factor (ETAF). J Immunol 127:1493–1498, 1981

18. Yoshida J, Cohen S: Lymphokine activity in vivo in relation to circulating monocyte levels and delayed skin reactivity. J Immunol 112:1540–1547, 1974

19. Kasahara T, Hooks JJ, Dougherty SF, Oppenheim JJ: Interleukin 2-mediated immune interferon (IFN-γ) production by human T cells and T cells subsets. J Immunol 130:1784–1789, 1983

20. Zackheim HS: Cutaneous T cell lymphomas. Arch Dermatol 117: 295–304, 1981

21. Clemmensen OJ, Bendtzen K, Andersen V, Wulf HC, Niebur E, Thomsen K, Bendixen G: Lymphocyte function and chromosome aberrations in patients with early mycosis fungoides and parapsoriasis en plaques. J Invest Dermatol 81:308–313, 1983